

Molecular cloning of the *XRCC2* human DNA repair gene, which is similar to *RAD51* of *S. cerevisiae*. N. Liu, J.E. Lamerdin, *Y. Zhao, *M.J. Siciliano, A.V. Carrano, and L.H. Thompson. Lawrence Livermore Natl. Lab., Livermore, CA, *Univ. of Texas M.D. Anderson Cancer Center, Houston, TX

Isolation of genes that correct DNA repair deficiency in rodent mutant cell lines is a powerful way to identify the individual steps in pathways of DNA repair. A mutagen-sensitive hamster V79 cell line, *irs1* (1), was used in this study to isolate a human cDNA that influences the response to DNA damage. The human gene that corrects *irs1*'s hypersensitivity was identified and assigned to human chromosome 7q36 by using somatic cell hybrids of *irs1* and human lymphocytes. This gene was defined as *XRCC2* [i.e. x-ray repair cross complementing (2)]. A cDNA expression library was transfected into *irs1* cells, and two transformants (I-PT4 and I-PT5) were obtained that showed ~10-fold more resistance to mitomycin C (MMC) and >4-fold more resistance to hygromycin B compared to *irs1*. The transformants were also partially corrected for sensitivity to cisplatin and ethyl methanesulfonate. One of the transformants (I-PT5) displayed a high instability of resistance to MMC and hygromycin. Southern blot analysis showed that the sensitive subclones of I-PT5 had lost the hygromycin gene sequence, which was retained in the resistant subclones. These results suggested that the acquired cDNA in I-PT5 was not integrated into the genome, although episomal replication of EBV-derived vectors in rodent cell lines has not been reported. A plasmid (pEBS-XR2) with a cDNA insert of ~3 kb was recovered from Hirt extracts of MMC-resistant subclones of I-PT5. This insert was subcloned into the pcDNAIII expression vector (Stratagene) resulting in the construct pcDNA-XR2. Both pEBS-XR2 and pcDNA-XR2 corrected *irs1* cells to MMC resistance upon electroporation. The *XRCC2* cDNA was localized to 7q36 by Southern blotting of a hybrid clone panel, confirming that the cDNA matches the *XRCC2* gene that complemented in hybrids. Incomplete sequencing data on the cDNA shows that *XRCC2* protein has significant similarity with the *Saccharomyces cerevisiae* DNA repair protein Rad51, which controls double-strand break repair. (Work was done under the auspices of the US. DOE by LLNL under contract No. W-7405-ENG-48.)

1. Jones et al, Mutat. Res. 183, 279-286, 1987,
2. Jones et al, Genomics 26, 619-622, 1995